# Regeneration of the long bone after implantation into its defect of osteoplastic material «Cerabone®»

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## SOUHRN

Korenkov A.:

## SUMMARY

Korenkov A.: **Regeneration of the long bone after implantation into its defect of osteoplastic material «Cerabone®»**
**Objective:** This work is aimed at the morphological study of the dynamics of healing of compact bone tissue defects after implantation into its cavity of «Cerabone®».
**Materials and Methods:** The experiment was conducted on 24 Wistar rats. In the middle third of the femoral diaphysis we created perforated defect to the medullary canal in the diameter of 2.5 mm, into which the osteoplastic material «Cerabone®» was implanted. Fragments of the injured bones were examined on the 60th and the 120th day with the methods of light microscopy with morphometry and scanning electron microscopy.
**Results:** The experimental study found that osteoplastic material «Cerabone®» in the area of the defect does not cause any inflammatory reaction, and adjacent to the site of implantation maternal bone there are lacunae with typical osteocytes. In the area of the defect lamellar bone tissue with a high content of osteoblasts and osteocytes was formed directly on the surface of «Cerabone®». The number of test material in the area of its implantation within the duration of the experiment remained almost unchanged.
**Conclusions:** Osteoplastic material «Cerabone®» in the area of compact bone tissue defect shows high biocompatibility, osteoconductive properties and ensures the stability of defect volume due to the good integration with bone tissue and absence of significant signs of resorption throughout the period of the experiment.

*Keywords: bone, hydroxyapatite, reparative osteogenesis.*

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## Introduction

One of the major problems faced by podiatrist in their practice is the tissue regeneration in the area of bone defects, which determines the need to find tools that would ensure their complete healing. [1]. Transplantation of the bone tissue to replace bone defects has a long history, during which there has been achieved considerable success. However, because of the high incidence of complications that may arise after such operations, the interest in the calcium-phosphate osteoplastic materials has increased in recent years [2]. The similarity of the chemical structure of such materials to bone tissue and relative inertness to biological tissues make it possible to widely use these to replace the lost bone tissue [3,4].

Osteoplastic drug from the bovine bone «Cerabone®» appeared on the market since January 2002. For this time it has demonstrated relatively high safety as to allergic reactions, bovine spongiform encephalopathy and other infections. This due to the fact that in the course of its production it is subjected to deproteinization by calcination at a temperature of 1 200 C [5]. «Cerabone®» and other drugs based on bovine hydroxyapatite proved themselves as osteoplastic materials that are well integrated into the newly formed bone tissue [6,7] and provide excellent long-term stability of the defect volume because of the very slow resorption [8,9]. However, studies, on the basis of which the above facts were received, were held at the cancellous bone, and information on the impact of osteoplastic material «Cerabone®» on the dynamics of the healing of the defect of compact bone tissue in the scientific literature has not been found. Most studies, devoted to the studies of the «Cerabone®», are concerned with morphological studies, which lack morphometric characteristics of histological preparations and microscopic, especially the electron-microscopic characteristics of the tissue-specific structures of regenerate. Therefore the aim of our study was to investigate the healing process of the compact bone tissue defect after implantation of osteoplastic material «Cerabone®» using histological, morphometric and electron microscopic techniques.

## Materials and Methods

The experiment was performed on 24 white Wistar rats eight months of age with a mean mass of 250 ± 10 g. All procedures were agreed with the Commission on Biomedical Ethics of Sumy State University (Minutes 4/13 of 05.18.2015). The study protocol was in accordance with the provisions „European Community Directive of 24 November 1986 on the maintenance and use of laboratory animals for research purposes“. Before surgery, animals were initially injected with 0.6 mg of acepromazine (2.5 mg per 1 kg body weight of rat), and in 5 minutes 18 mg of ketamine (75 mg per 1 kg of rat weight). After the introduction of the animals in anesthesia, we drilled, under aseptic conditions, the defect to the medullary canal with the diameter of 2.5 mm, this in the middle third of the femoral shaft, using a portable drill with a spherical cutter at low speed with cooling, and without rigid fixation, we filled it with the osteoplastic material «Cerabone®» (Botiss, Germany registration number 2012/12810 FES). It is a natural hydroxyapatite of spongy substance of the tubular bones of cows in the form of granules (2 mm, Ca/P-1.67), which have a three-dimensional pore structure (pore size is 200–350 microns) rough and hydrophilic surface (Fig. 1) [10]. In addition, 1 g of «Cerabone» contains about 1 mg of bone morphogenetic protein-2, with the regulatory action of which the adequacy of osteogenesis, osteogenic differentiation of precursor cells into osteoblasts is associated [11].

Before the implantation the granules of «Cerabone®» were moistened in own blood of a rat, which was taken from the tail vein to fill the pores and remove residues of air from the material and also to provide more plastic consistency of the material. After entering into the bone defect of osteoplastic material, the wound was tightly stitched with silk thread through all layers of soft cover, the seam was treated with 3% alcohol solution of iodine. Then, during the next 3 days after operation for prevention of septic complications the after-operation seam was treated with an alcohol solution of iodine, and for analgesia ketorolac was injected intramuscularly at a dose of 0.6 mg 2 times a day.

After 60 and 120 days after surgery the animals were taken out of the experiment by decapitation under deep ether anesthesia with further research of the injured bones by the methods of light (with morphometry) and scanning electron

Figure 1
The microstructure of osteoplastic material «Cerabone®». One can see the pores ranging in size from 67 to 323 microns. Electronic scanning image. X 120 magnification

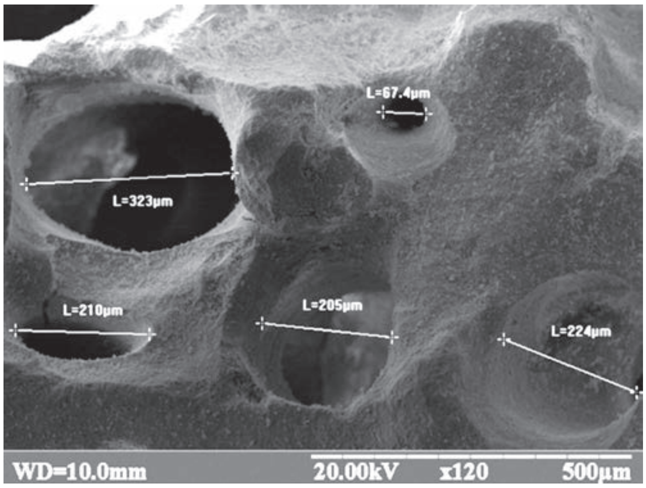
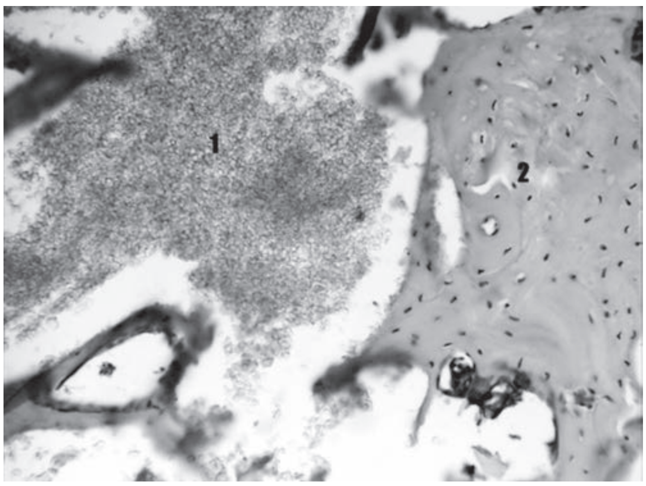



Figure 2
The area of the femur defect of a rat on the 60th day after the implantation of «Cerabone®». Remains of osteoplastic material (1) integrated into lamellar bone tissue of the regenerate (2). Haematoxylin and Eosin staining. X 200 magnification



microscopy, which was performed on the electron microscope „REM 106-I“. Histological cuts stained with hematoxylin and eosin, were analyzed in light microscope «OLYMPUS», photographed with a digital camera. Morphometric analysis was performed using image processing program „Video-Test“ and „Video-Size“ [12]: we determined in the area of the defect the relative percentage of bone tissue and remnants of osteoplastic material as the ratio of the area of these components (%) to the total area of the site of the defect (100 %). In addition, we investigated the state of the structure of the adjacent to the site of implantation maternal bone in order to establish or refute postoperative complications due to the presence or absence of signs of necrobiosis and necrosis of osteocytes [13]. The obtained values were processed statistically calculating the mean value (M) and standard error (SE). The significance of differences between comparable indicators was evaluated using Student t-test with the use of statistical computer program MS Excel XP. The differences were considered significant at $p < 0.05$ [14].

Figure 3
The area of the femur defect of rat on the 60th day after the implantation of «Cerabone®». Secondary osteoblasts (1) and osteocytes (2) in the bone tissue, which was formed directly on the surface of osteoplastic material. Electronic scanning image. X 3000 magnification

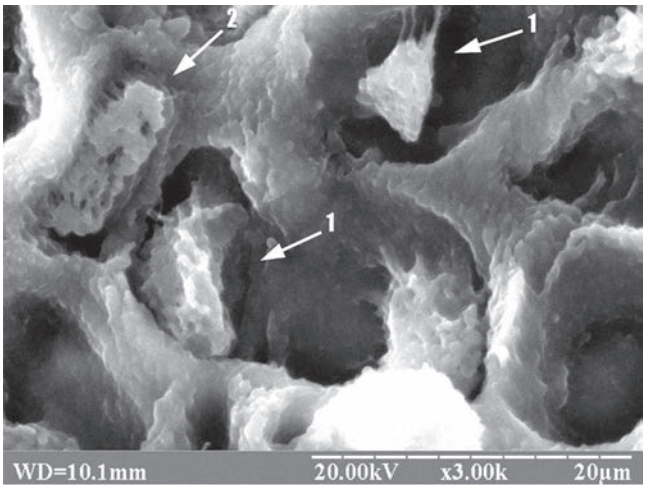
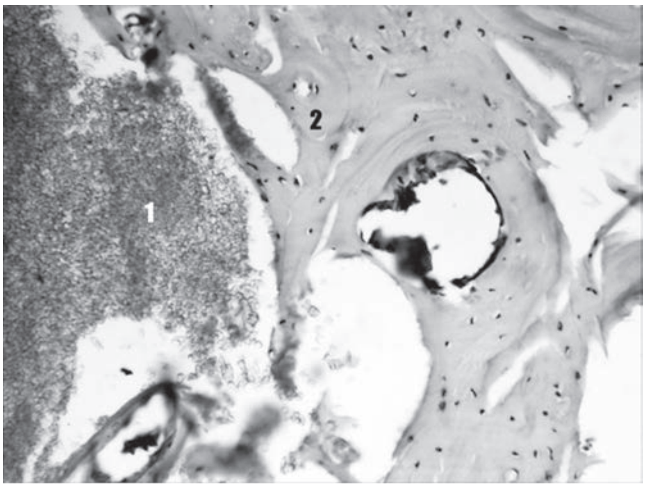



Figure 4
The area of the femur defect of a rat on the 120th day after the implantation of «Cerabone®». Remains of osteoplastic material (1) integrated into lamellar bone tissue of the regenerate (2). Haematoxylin and Eosin staining. X 200 magnification




## Results

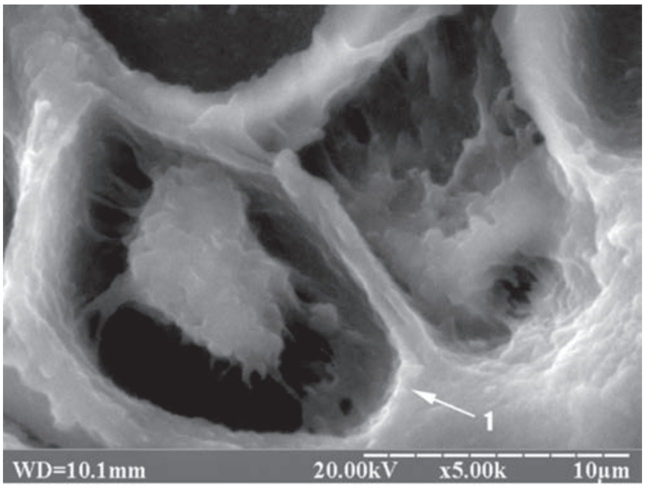
On the 60th day of the experiment the site of the defect without evidence of inflammation was filled with newly-formed bone tissue of regenerate and integrated into its structure remains of «Cerabone®». Here, osteoplastic material and bone tissue occupied 40.81 ± 1.69% and 59.19 ± 1.69 % of the total area of the defect, respectively. At the time of observation the connective tissue in the area of the defect was not detected, and the bone tissue of the regenerate had a lamellar structure and contained in its composition secondary osteoblasts and osteocytes (Fig. 3). The formation of bone tissue with the generated osteons occurred directly on the outer surface of the «Cerabone®», but inside the remnants of the implanted material osteogenic cells and foci of osteogenesis were not found. As part of the parent bone adjacent to the implant site of «Cerabone®» there were observed typical osteocytes with long spikes.

On the 120th day of the experiment a significant portion of the defect was occupied by the remains of osteoplastic material «Cerabone®», which after hematoxylin-eosin staining had a greenish color and were fully integrated into the newly formed bone tissue of regenerate (Fig. 4). The latter was formed directly on the outer surface of the implant, had a lamellar structure and contained in its composition a significant amount of secondary osteoblasts and osteocytes (Fig. 5). In this case, inside the residues of osteoplastic material as on the 60th day of the experiment, there were found neither osteogenic cells nor osteogenic foci. The area, which was in the area of the defect occupied by the osteoplastic material was 38.78 ± 1.48 %, while area occupied by bone tissue was 61.22 ±1.48 %.This was insignificantly less ($p > 0.05$) in the first and greater ($p < 0.05$) in the second case compared with the previous period of observation. Signs of inflammation in the area of implantation of osteoplastic material «Cerabone®» were not found, and in structure of the parent bone, as in the previous observation period, there were typical osteocytes.

## Discussion

The performed study found that the osteoplastic material «Cerabone®» has high biocompatibility, as evidenced by the absence of inflammatory reaction in the area of the defect and the presence of parent bone of lacunae with typical osteocytes adjacent to the site of implantation. Furthermore, «Cerabone®» in the area of the defect showed osteoconductive properties, and also good integration with the bone tis-

Figure 5
The area of the femur defect of a rat on the 120th day after the implantation of «Cerabone®». Osteocytes in a lacuna of bone tissue of regenerate, which is formed directly on the surface of osteoplastic material. Electronic scanning image. X 5000 magnification




sue of regenerate as evidenced by tissue growth directly on the outer surface of osteoplastic material. In this case, at 60–120th day after implantation of «Cerabone®» the newly formed bone tissue was mature as evidenced by formed osteons and also typical secondary osteoblasts and osteocytes in its composition. It should also be noted that throughout the whole period of the experiment the number of osteoplastic material «Cerabone®» in the area of its implantation remained almost unchanged. Research work of other authors report on a slow resorption of osteoplastic materials from bovine bone, but it has been so far demonstrated on the cancellous bones and jaws. Mordenfeld et. al. and Sartori et. al. [15, 16] discovered the remains of osteoplastic material in the upper jaw of people in 10–11 years after its implantation. At the same time using histological and morphometric analysis they showed that the rate of resorption of the implant and its replacement by a newly formed bone tissue is 3.55 % per month in the first two years and 0.58 % in subsequent years. In our study, we did not observe any reliable signs of resorption of «Cerabone®» in the defect of diaphysis of the femoral shaft of rats. This fact was confirmed by measuring the residual area of osteoplastic material that between the 60th (40.81 ± 1.69 %) and 120th (38.78 ± 1.48 %) day almost did not change, which enabled using «Cerabone®» to ensure the stability of volume of the defect throughout the period of the experiment.

## Conclusions

Thus, osteoplastic material «Cerabone®» in the area of the compact bone tissue defect shows a high biocompatibility, osteoconductive properties and ensures the stability of the volume of the defect due to the good integration with bone tissue of regenerate and the absence of reliable evidence of resorption on the whole period of the experiment.

## References

1. Zacepin ST. Bone pathology of adults. Guide for physicians. Medicine. Russia, Moscow 2001; p 450.
2. Germanov VG, Kovalersky GM, Cherkashena ZA, Semenov VA. Osteoplastic surgery: From bone transplant to the modern biocomposite materials. Medical Assistance Available 2006;4:16–19.
3. Barinov SM, Komlev VS. Bioceramics based on calcium phosphates. Nauka. Russia, Moscow 2005; p 308.
4. Pankratov AS, Lekishvili MV, Kopetsky IS. Bone grafting in dentistry and maxillofacial surgery. Osteoplastic materials: A Guide for Physicians. BINOM. Russia, Moscow 2011; p 272.
5. Murugan R, Panduranga K, Sampath Kumar RAO, Sampath Kumar TS. Heat-deproteinated xenogeneic bone from slaughterhouse waste: Physico-chemical properties. Bull Mater Sci 2003;26(5):523–528.
6. Artzi Z, Wasersprung N, Weinreb M, Steigmann M, Prasad HS, Tsesis I. Effect of guided tissue regeneration on newly formed bone and cementum in periapical tissue healing after Endodontic Surgery: an in vivo study in the cat. J Endod 2012;38(2):163–169.
7. Rothamel D, Schwarz F, Smeets R, Happe A, Fienitz T, Mazor Z, Zöller J. Sinus floor elevation using a sintered, natural bone mineral – A histological case report study. J Dent Implant 2011;27(1):60–70.
8. Huber FX, Berger I, McArthur N, Huber C, Kock HP, Hillmeier J, Meeder PJ. Evaluation of a novel nanocrystalline hydroxyapatite paste and a solid hydroxyapatite ceramic for the treatment of critical size bone defects (CSD) in rabbits. J Mater Sci 2008;19(1):33–38.
9. Riachi F, Naaman N, Tabarani C, Aboelsaad N, Aboushelib MN, Berberi A, Salameh Z. Influence of material properties on rate of resorption of two bone graft materials after sinus lift using radiographic assessment. International Journal of Dentistry 2012;2012:1–7.
10. Tadic D, Epple M. A thorough physicochemical characterisation of 14 calcium phosphate-based bone substitution materials in comparison to natural bone. Biomaterials 2004;25:987–994.
11. Seidel P, Dingeldein E. Cerabone® – eine Spongiosa-Keramik bovinen Ursprungs. Materialwissenschaft und Werkstofftechnik 2004;35(4):208–212.
12. Sarkisov DS, Perov YL. The microscopic technique. Medicine. Russia, Moscow 1996; p 542.
13. Grigorian AS, Toporkova AK. Problems of integration of implants in bone tissue (theoretical aspects). Technosphere. Russia, Moscow 2007; p 128.
14. Lapach SN, Hubenko AV, Babich PN. Statistical methods in biomedical research using Excel. Morion. Ukraine, Kiev 2000; p 320.
15. Mordenfeld A, Hallman M, Johansson CB, Albrektsson T. Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. Clin Oral Implants Res 2010;21(9):961–970.
16. Sartori S, Silvestri M, Forni F, Icaro Cornaglia A, Tesei P, Cattaneo V. Ten-year follow-up in a maxillary sinus augmentation using anorganic bovine bone (Bio-Oss). A case report with histomorphometric evaluation. Clin Oral Implants Res 2003;14:369–372.